Enzyme characterisation in microreactors by multivariate data analysis

Ringborg, Rolf Hoffmeyer; Krühne, Ulrich; Woodley, John

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

ENZYME CHARACTERISATION IN MICROREACTORS BY MULTIVARIATE DATA ANALYSIS

Rolf H. Ringborg [1] – Ulrich Krühne [1] – John M. Woodley [1]

[1] Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark, E-mail: Rolri@kt.dtu.dk

Keywords: Enzymes, Microreactor, UV, spectroscopy, Enzyme Characterization, Enzyme Kinetics.

Characterization of new enzymatic mutants is currently time consuming and requires a high degree of knowledge to understand and interpret the enzymatic performance correctly. Clearly, it would be good to accelerate the development of biocatalysis for industrial use and one way to do that is by rapid characterization. As an example ω-transaminase is here investigated, which facilitates the exchange of an amine- and keto-group stereoselectively, see Figure 1.

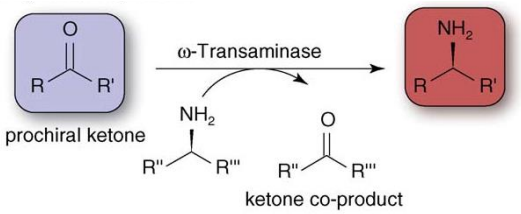

Figure 1 - ***Asymetric synthesis of prochiral ketones [1]***

Enzyme characterization is considered in the form of taking a picture, this will include studying the effect on initial rate of pH, Enzyme, Substrate, co-Substrate, Product and co-Product concentration [2]. From this investigation, it will be possible to determine whether the enzyme meets the criteria for scale-up or not. The characterization will be carried out in a microreactor [3], this size is ideal since the enzyme resource is scarce at this point of development and currently the only concept that facilitates this analysis. It will therefore be possible to investigate the biocatalyst thoroughly with only small quantities of enzyme consumed. In the case where the reaction operates with UV active components, UV can be used to detect compounds with high sensitivity supplemented by multivariate data analysis where the spectra can be decorrelated to yield concentrations of individual compounds. HPLC systems are built for handling small quantities of liquids and the UV detectors for these proves to be fitting excellent. Enzyme characterization will therefore be carried out by combination of a microreactor with a diode array detector from an HPLC system.

References:

1. D. Koszelewski, K. Tauber, K. Faber and W. Kroutil, "ω-Transaminases for the synthesis of non-racemic a-chiral primary amines" Trends in Biotechnology, 28, pp. 324-332, 2010

2. N. Al-haque, P. A. Santacoloma, W. Neto, P. Tufvesson, R. Gani and J. M. Woodley, "A Robust Methodology for Kinetic Model Parameter Estimation for Biocatalytic Reactions" Biotechnol. Prog., 28, No. 5, pp. 1186-1196, 2012

3. U. Krühne, S. Heintz, R. Ringborg, I. P. Rosinha, P. Tufvesson, K. V. Gernaey and J. M. Woodley, "Biocatalytic process development using microfluidic miniaturized systems", Green Process Synth., 3, pp. 23-31, 2014